RELEASE OF PARASITOIDS TO CONTROL GREENBUGS ON SORGHUM

ARS-S-91
April 1976

AGRICULTURAL RESEARCH SERVICE • U.S. DEPARTMENT OF AGRICULTURE

CONTENTS

TABLES

RELEASE OF PARASITOIDS TO CONTROL GREENBUGS ON SORGHUM

By K. J. Starks,[1] R. L. Burton,[1] G. L. Teetes,[2] and E. A. Wood, Jr.[1]

ABSTRACT

As an alternative to insecticides for greenbug control on grain sorghum, *Lysiphlebus testaceipes* (Cresson), a native parasitoid, was reared on plants infested with greenbugs, *Schizaphis graminum* (Rondani), in greenhouses at Stillwater, Okla.; acclimatized on plant material at 5° C for storage; and shipped by air as mummies to Lubbock, Tex., for release in sorghum fields when greenbug populations were below 12 per plant. Emergence of adults was better than predicted, but release points should have been closer together. In plots where 14,000 and 28,000 parasitoids were released per acre, greenbugs did not increase to damaging populations as rapidly as they did in releases of 7,000 parasitoids per acre and in control plots. Counts of *L. testaceipes* mummies indicated higher numbers in plots where parasitoids were released. Parasitoid releases at 14,000 and 28,000 per acre along with insecticide applications resulted in grain yields significantly higher than produced in control plots. *Aphelinus asychis*, an imported parasitoid of aphids, and native predators were not effective in preventing greenbug damage. Corn leaf aphids, another host of *L. testaceipes*, did not help in the establishment of *L. testaceipes*.

INTRODUCTION

The greenbug, *Schizaphis graminum* (Rondani), has been the major pest of small grains in the Central and Southwestern States since the turn of the century, but, until recently, has usually caused extensive damage in a given area only about every 6 to 8 years (*8*).[3] The insect was thought to be a cool weather pest and to depend on native grasses (nonpreferred host plants) for summer carryover of small populations. Then, in 1968, a variant designated as biotype C (taxonomically similar to but physiologically different from previously known biotypes in the United States) became a major pest on grain sorghum. At present the greenbug can build up large populations in both dryland and irrigated sorghum and can withstand relatively high temperatures (*15*). Instead of sporadic outbreaks of greenbugs, some areas now have a chronic problem.

Many varieties of commercial feed barley are resistant to greenbugs, and chemical control is not necessary. Chemicals associated directly or indirectly with resistance in barley have been identified (*4*), but the practical significance of these has yet to be demonstrated. Other small grains have no commercially acceptable level of greenbug resistance. Varieties of resistant wheat were about to be released, but when biotypes B and C became prevalent, all known wheat selections indicated susceptibility. Ryes resistant to greenbugs should be available commercially soon. Triticale, which incorporates resistance from 'Insave F. A.' rye, is promising, but will not be available commercially for several years. Several excellent sources of resistance have been found in sorghum, and these will be available in commercial hybrids in 1975 (*2*, *13*). It may be several years before plant resistance is applicable on a large scale. Therefore, the

[1]Research entomologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Entomology, Oklahoma State University, Stillwater, Okla. 74074.

[2]Associate professor, Texas A&M University Agricultural Research and Extension Center, Route 3, Lubbock, Tex. 79401.

[3]Italic numbers in parentheses refer to items in "Literature Cited" at the end of this publication.

only current, practical, applied control of greenbugs on sorghum and small grains except barley is insecticides.

Insecticides have provided good control of greenbugs on small grains with comparatively small, inexpensive dosages. For example, parathion at 0.25 pound AI per acre has been applied aerially to wheat for about 20 years. The greenbug can become damaging to small grains when temperatures are too low for insecticides to be effective. Insecticides are being used on sorghum, increasing production costs roughly $18 million a year for greenbug control. Generally, ethyl parathion or disulfoton is used at recommended dosages of 4 to 8 and 4 to 6 ounces AI per acre, respectively. Control on small grains and sorghum was usually near 100% until 1974, when organophosphorus insecticide resistance occurred (*6*, *11*). Also, the Banks grass mite, *Oligonychus pratenis* (Banks), formerly no problem, has become highly resistant to some insecticides and is now a serious pest of both sorghum and corn (*12*). Furthermore, many insecticides kill predators that could keep the Banks grass mite and other pests at economically inconsequential populations.

The cost of chemical control, the resistance of the greenbug and the Banks grass mite to insecticides, and the killing of beneficial predators and parasites by insecticides warrant alternatives for controlling greenbugs. Host plant resistance seems to be the most practical long-range alternative, but farmers need as many control options as possible. Release of parasitoids was studied as a possible control of greenbugs on sorghum.

PARASITOIDS AND PREDATORS OF GREENBUGS

Diseases of the greenbug are rare and little studied. However, several parasitoids and predators of the greenbug have been reported. Some of these are not native to the United States, and considerable research on basic aspects would be necessary before they could be considered for a release program. Others are native and their relationships with the greenbug are better known, though not completely. Native predators are often general feeders and transients, and they take so long to complete a generation that greenbugs outproduce them. Lady beetles, mainly *Hippodamia convergens* Guérin, are usually the most conspicuous predators of greenbugs, and both larvae and adults can consume large numbers. Nevertheless, importing this species commercially from California and releasing it in sorghum has not been beneficial since the released adults leave the area promptly (*10*).

Native parasitoids are more restricted to aphids but are also represented by fewer species. *Aphelinus nigritus* (Howard) can become numerous in greenhouses but is scarce in fields (*14*). *Lysiphlebus testaceipes* (Cresson), a braconid, seems to have followed the spread of the greenbug throughout its range in the United States and prefers this aphid as a host, although other small-grain and sorghum aphids can be parasitized. Thus, *L. testaceipes* is well adapted. It has good reproductive potential (*3*), can spread either directly as adults or indirectly as larvae within winged aphids, and can even withstand a certain amount of chemical control if subjected to insecticides during the proper mummified stage (*5*). The adults of *L. testaceipes* can be highly active on plants and readily oviposit in greenbugs, resulting in large numbers of golden-colored mummies. Even so, there are some important factors that prevent *L. testaceipes* from being the ideal parasitoid:

1. The greenbug, compared to *L. testaceipes*, can reproduce at lower temperatures. This fact can result in the greenbug "getting the jump" on its parasitoid in the spring.
2. The greenbug usually takes 6 to 10 days to complete a life cycle, whereas *L. testaceipes* needs about 3 or 4 days longer to go from newly laid egg to ovipositing female. Therefore, the pest can quickly get ahead of its enemy during early generations.
3. Some hymenopterous secondary parasitoids frequently become abundant just when *L. testaceipes* seems to have a greenbug infestation under control. This probably could result in an explosion of greenbugs while *L. testaceipes* is left behind.
4. Present insecticidal treatment returns a field to the early spring condition—that is, the greenbug, with its shorter life cycle, can reinvade a field and become abundant before the lagging parasitoid can catch up. Consequently, repeated insecticidal treatments or else long residual treatments, especially on sorghum, are needed.

GENERAL RELEASE PROCEDURES

The High Plains of Texas were chosen as the parasitoid release area because of the extensive growing of grain sorghum and the high infestation of greenbugs each year since 1968. In 1972 permis-

sion to release parasitoids was obtained from growers in two counties on about 9,900 acres. Difficulties in rearing parasitoids and other problems prevented scheduled inoculation releases. Since there were no provisions for reimbursing growers for losses and there was a lack of professional help to adequately discuss the program with individual growers, the program was not very successful. However, valuable experience and information were gained, and many of the same technicians trained then were used again in 1973.

We obviously needed preliminary, smaller tests and better control of experimental plots. Therefore, cooperative agreements were made in 1973 with two progressive sorghum growers, and sorghum was leased from the Texas A&M University Agricultural and Extension Center at Lubbock. About 180 acres of irrigated sorghum were used. The fields were divided into plots of about 10 acres for each of six treatments. The treatments were planned as follows: (1) no parasitoids or insecticide, (2) release of 7,000 parasitoids per acre, (3) release of 7,000 *Aphelinus asychis* adults per acre (rearing of *A. asychis* was not satisfactory so 14,000 *L. testaceipes* per acre were substituted), (4) release of 28,000 *L. testaceipes* per acre, and (5) disulfoton treatment. There were three replications of each treatment. Cultural practices conducive to good yields were used; these and the selection of commercial hybrids were left to the choice of the producer. Fortunately, one of the cooperators planted early (April 24–25) and the other one planted late (May 25–29). This helped distribute the need for parasites over a longer period of time and thus made rearing them easier. Planting was in 40-inch rows with about one-half of the area in single rows and the other half in double rows. Plant population varied among the 10-acre plots from about 42,600 to 94,700, but this should not have influenced the results of the test to any measurable extent.

REARING, STORING, AND SHIPPING PARASITOIDS

This is the first research project to attempt mass production of *Lysiphlebus testaceipes*; rearing was previously restricted to small pots with susceptible barley to rear the host greenbugs. It was obvious that synchronization of plants, greenbugs, and parasitoid life cycles would be a problem. Rearing procedures in 1973 were similar to those used in 1972, but some techniques were discontinued. For example, nutriculturing of plants was not reliable, mass inoculation of cultures needed refinements, and packaging and shipping of adults presented too many unsolved problems. Methods used in 1973 were simpler, but not as efficient as those used in 1972. Since fewer parasitoids were needed in 1973, production was reduced, and more time was available for trying different techniques. One other factor that made rearing much easier in 1973 was successful mummy storage, which allowed for stockpiling of parasitoids until needed.

Rearing

Three separate greenhouse rooms were required for growing host plants, rearing aphids, and producing parasitoids. If possible the temperature was maintained between 25° and 30° C, with an average relative humidity of about 60%. Separate rooms were necessary to prevent premature infestation before plants were large enough to support greenbug colonies and to prevent untimely parasitization of aphids. Untimely parasitization kept greenbug populations from attaining optimum numbers and produced mummies of different ages. All plantings were caged before seeds germinated to assure production of clean colonies, i.e., free of secondary parasitoids. Plants growing outside the caged area were removed. Cages were constructed from clear cellulose plastic nitrate, 0.05 centimeter thick, and fastened at the edges with a ketone-base glue. For aeration, several holes were cut in the plastic before construction; the holes and the tops of the cages were covered with thin muslin. The aeration was sufficient to prevent condensation of moisture within the cages, which would trap the insects.

For aphid cultures, a mixture of susceptible 'Rogers' barley and 'Wheatland' sorghum was planted in aluminum flats and plastic pots (50 flats and 100 pots weekly). The barley furnished a preferred host for quick establishment of the greenbugs, and the sorghum was better able later to withstand feeding by large numbers of greenbugs. The barley was treated with Cerasan and the sorghum with Captan before planting to discourage "damping off." Upon germination the plants were given a solution of Hyponex plant food (7–16–19 analysis) at the rate of 5 grams per gallon of water.

Ten days after planting the plants were transferred to the greenbug culture room and manually infested at the rate of 1 or 2 greenbugs per plant or about 400 per flat and 25 per pot. These figures were determined in the laboratory by the following replicated test: 'Wheatland' sorghum was seeded in plastic pots and thinned to two plants when seeds

emerged. Ten days after seeding, the plants were infested at the rates of one, two, three, four, and five adult greenbugs per plant with five replications and were caged. Ten days after infesting, greenbug counts were taken, and one mated female parasite per plant was released within each cage. Ten days later the plants were rated for damage, and mummified aphid counts were taken. These results are summarized in table 1. When a 10-day interval between infesting and parasitizing was used, more than three greenbugs per plant resulted in death of the plant, which in turn destroyed both aphids and parasitoids. Therefore, either the number of days before parasitization should be reduced or only one or two greenbugs per plant should be the rate of infestation. As adult parasitoids rarely live more than 48 hours, parasitization must occur during that short interval.

TABLE 1.—*Effect of greenbug infestation rate on parasite production*

Greenbug infestation rate (No./plant)	Greenbugs/plant 10 days after infestation	20 days after infestation	
		Plant injury[1]	Mummies/ plant[2]
1	52	4.0	22.0
2	76	4.8	14.0
3	98	5.0	1.6
4	152	6.0	.0
5	103	6.0	.0

[1]Scale of 1 to 6: 1=no injury to plant; 6=dead plant.
[2]Parasitoids introduced 10 days after greenbugs.

Ten days after infestation, the plants were moved to the parasite culture room where about 300 unsexed adult parasitoids were released per flat and about 25 per pot. Usually 8 to 10 flats were saved to collect parasitoids for the subsequent test; emergence of these parasitoids could be delayed several days by storing the flats at 5° C. When ready for use, these flats were returned to the parasitoid room and entirely covered with a black cloth. Two 12-dram vials were screwed into holes drilled into a triangle of plywood nailed to opposite corners at the top of each cage. Holes cut in the cloth were fitted over the vials. Because the parasitoids are phototrophic, they were attracted to the light and captured in the vials. Parasitoids were also captured with aspirators from caged, potted plants infested and parasitized to coincide with the succeeding test. A pot label in each flat recorded the test series, flat number, and date of infestation and parasitization.

If *L. testaceipes* does not have access to plants and greenbugs where nectar and honeydew are available, then food must be provided. Table 2 shows the reduction in mortality when food, especially fructose, is available to the parasite. Testing showed that feeding for only 24 hours did not improve longevity, indicating that continuous feeding is necessary.

TABLE 2.—*Percentage of live* L. testaceipes *fed various sugars*

Sugar fed	Percentage of live adults on days after emergence[1]			
	1	2	3	4
Fructose	88	60	35	25
Glucose	55	53	45	23
Sucrose	90	80	58	10
Raffinose, fructose, glucose, and galactose combination	95	75	50	20
Water only	85	10	0	0
No liquid	60	5	0	0

[1]Initially, there was a total of 40 adults in each treatment.

Storing

The plants with parasitized aphids were harvested 8 to 9 days after parasitization and placed in cold storage. Several types and sizes of storage-shipping boxes were tried, but a corrugated paper container measuring 2 by 2 by 4 inches was used. Random mummy counts gave an estimate of about 5,000 parasitoids per flat. A few mummies were taken from each flat and placed in 4-dram vials for the detection of secondary parasitoids. *Pachyneuron siphonophorae* (Ashmead) was a constant threat, but careful selection and use of only adults for parasitizing kept its numbers low. During harvest, many parasitized aphids were dislodged from the plants, but were recovered on uncaged culture pots placed in the flats. Within several hours the heavily infested plants were caged. This method provided a constant source of parasitoids for succeeding tests.

Preliminary tests were done to determine the best age for storing, the effect of time on mortality, and the effects of acclimatization on survival. In one experiment 100 parasitized greenbugs were placed in each of 45 petri dishes. Five replications of greenbugs parasitized for 5, 7, and 9 days were placed in a walk-in refrigerator maintained at 5° C in total darkness. These were removed at intervals of 10, 20, and 30 days, and parasitoid emergence

counts were made. The effects of acclimatization were studied in another replication placed at 16° C for 12 hours before being stored at 5° C.

Table 3 shows that 5-day-old mummies acclimatized at 16° C produced the most parasitoids after 30 days of storage. Nine-day-old mummies produced the most parasitoids if removed from storage within 10 days. Therefore, the mummies were harvested 8 or 9 days after parasitization, acclimatized, and shipped as soon as possible.

TABLE 3.—*Effects of storage time at 5° C and mummy age on emergence of* L. testaceipes

Days after greenbug parasitization	Percentage of emergence after—			
	10 days	20 days	30 days	30 days[1]
5	9	12	5	51
7	43	44	10	43
9	81	14	10	12

[1]Acclimatized for 12 hours at 16° C before storage at 5° C.

Mechanical removal of mummies from plants was tried, but survival of parasitoids was unsatisfactory, presumably because of desiccation. Therefore, mummies were stored attached to dried plants. It was seldom necessary to dry plants beyond 1 or 2 hours before packaging.

Because the parasitoids were not subjected to light until they were released, there was some speculation about the occurrence of mating in the dark. There was some apprehension that parasites escaping the release boxes in the field would have difficulty locating the opposite sex, and mating would not occur. Unfertilized females parasitize their host, but the resulting progeny are all males. Under normal conditions the ratio of males to females is about 1:1 (*3*). A test was therefore designed to determine the effects of darkness and the availability of food and water on this ratio.

Ten pots were seeded with barley and sorghum and infested with 100 greenbugs 10 days later. Two release boxes containing mummies maintained at 5° C for 7 days were removed from refrigeration, and release holes were cut from four corners of their lids. Two cylindrical sponges were glued to the inside lid of each box. The sponges of one box were treated with 1 cubic centimeter of tapwater, and the sponges of the second box were impregnated with 1 cubic centimeter of 20% fructose solution. The boxes were then sealed, and 12-dram vials were taped over the release holes. The parasitoids were captured in the vials immediately upon emergence, before mating could occur. Two females from the box with water were placed in each of five culture pots, and two females from the boxes containing fructose were placed in each of five culture pots. Nine days later the plants having mummies were harvested and placed in cylindrical ice cream cartons. The emerging parasitoids were captured in vials, counted, and sexed.

Table 4 shows that darkness had little effect on the female:male ratio. A slight decrease in the number of females was noted when fructose was supplied as food; however, combining the two tests produced an average of 52 females, indicating that fertilization did occur in the dark.

TABLE 4.—*Effect of food and water on the fertility of adult parasitoids emerging from mummies stored in the dark*

Treatment	Avg. No.		Females emerged (%)
	Mummies formed	Parasitoids emerged	
Water	134	131	60
Fructose	144	119	44

Shipping

The storage and release boxes containing mummies on plant material were sprayed inside with a fine mist of water before sealing. Twelve of these boxes fit into an insulated corrugated-paper shipping box. Four frozen packets of Blue Ice coolant, each containing 0.9 liter of gel, were placed inside the shipping boxes near the top just before shipment by air freight to Lubbock, Tex. It took about 4 hours to transport the boxes. The shipping boxes maintained a temperature near 5° C for about 2 days and then gradually warmed to ambient temperature during the next 24 hours. This usually allowed ample time for shipment and release. If a longer holding period was needed, fresh packets of coolant could be substituted.

Shipment of the mummies was satisfactory, except that a few boxes had cold spots, probably from being too closely in contact with the coolant at the top of the box. Sometimes the flaps on release boxes were not pulled closely together and newly emerged adults became trapped on the sealing tape.

RELEASING PARASITOIDS IN FIELDS

In preparation for release, four small holes were cut in the top corners of the release box, each about one-eighth inch in diameter. The boxes were usually

allowed to gradually warm to room temperature and were put in the field the day of arrival or the morning after arrival. The boxes were placed on wooden stakes, marked with plastic flags, about 200 feet apart within fields, with one release per 0.4 acre. The boxes were secured to the stakes with rubberbands about 6 inches above the ground because observations indicated that the parasitoids are more efficient if released near the lower leaves where greenbugs are located. Adults, especially the first day after emergence, tended to locate in whorls but did not seem to be parasitizing the corn leaf aphid to any extent, although this aphid was abundant. Maybe the parasitoids were feeding. They were able to walk around on leaves, even though the wind on one day reached gusts up to 40 miles per hour.

Emergence started even before the boxes were put in the field, but there were few dead adult parasitoids in boxes. Emergence continued for about 3 days. A sample of 1,234 adults indicated that adults emerged from 71.7% of the mummies put into the fields. Of the adults that emerged, about 64% emerged within 16 hours after being placed in the field, about 89% emerged within 40 hours, and the remaining 11% emerged later. The boxes were removed 4 days after placement in the fields to prevent the introduction of secondary parasitoids, since these were often present in small numbers and usually developed 4 days or more after primary parasitoids.

Results from 1972 indicated that a parasitoid release level of 2,500 per acre with about 10,000 greenbugs per acre, or 1 or 2 per plant, was inadequate to give reliable infestations. Some of the reasons this release procedure gave less than satisfactory results will be discussed, but time did not permit adequate testing to eliminate any one factor. Also, the factors might have interacted.

First, environmental factors might have been adverse. The adult parasites might have lacked food; however, feeding before release was not shown to increase efficiency or longevity. Weather conditions varied, but this must be expected if the procedure is to be successful.

Second, mating would not be satisfactory if it did not take place before dispersal; however, tests indicated that this was not the case. Neither would mating be satisfactory if the sex ratio was not 1:1 but instead was predominantly males or almost entirely females. The females will lay eggs in the absence of males and fertilization, but the offspring will all be males.

Third, the parasitoid might not be as efficient as laboratory tests indicated. Released adults did not survive long enough to effectively parasitize greenbugs. Females survived about 2 or 3 days, and the males, a shorter time. This is about the same longevity obtained in the greenhouse. Also, adults did not move sufficiently far from the release points. To correct this factor, release stakes were located 50 feet apart, and this proved adequate.

Fourth, there was a density factor for greenbugs before search by parasitoids could be efficient or oviposition could be stimulated. This factor was not measured since an elaborate test would have been needed. However, it was noted that the average of one to two greenbugs per plant did not occur evenly on plants. Instead, there was likely to be a female with several young on a plant and an absence of greenbugs for several plants. Therefore, more greenbugs might be needed before releasing parasitoids. In 1973 the level of greenbugs per plant at the time of release was about 3.7 in one of the replications and 11.8 in the others. There were no natural infestations of *L. testaceipes* in any of the fields at the time of release.

DISTRIBUTION OF PARASITOID ADULTS AFTER RELEASE OF MUMMIES

The distribution of the adult parasitoids was studied in one of the three replications. The original plans were to release 5,000, 10,000, and 20,000 parasitoids per acre. However, the emergence was predicted at only 50% when actually there was about 72% emergence, based on counts of exit holes from mummies. Allowing for some mortality of adults that never escaped from the release boxes, the rates of release have been assigned at 7,000, 14,000, and 28,000 parasitoids per acre. Each area sampled for adults had 2,827 square feet containing about 5,100 plants. This means that roughly 28% of the adults could be accounted for in the sampled areas, but almost one-half of these had not spread 3 feet or farther from the release point within 48 hours. Assuming that the parasitoids dispersed in a concentric pattern from the release point, and considering that the release points were 200 feet apart, then the parasitoids would need to spread about 100 feet in each cardinal direction before overlapping from adjacent release points would occur. Also, adults would need to travel about 141 feet to reach all plants within the radius of the release point. The

data suggested that release points needed to be closer, but that the rate of release may have been adequate for *L. testaceipes* to establish early in the field, considering the results from field cage studies not reported in this paper.

We should have found twice as many parasitoids in the 14,000 parasitoids per acre plots as in the 7,000 parasitoids per acre plots, and four times as many in the 28,000 parasitoids per acre plots. This was not the case; there were less than three times as many in the 28,000 per acre plots than in the 7,000 per acre plots. The reason for this is unknown.

The spread of the parasitoids in directions was amazingly uniform (table 5). The wind directions and speeds from the time of release until counts were made were changeable, but the strongest wind was from the north, gusting up to about 40 miles per hour about 36 to 40 hours after release started. Either this effect was counteracted by wind blowing from other directions or else wind has little effect on the spread of *L. testaceipes*. The latter possibility is supported by observations. There was some indication that the adults moved up and down rows since these ran east and west.

INSECT POPULATIONS IN RELEASE TEST

Five samples were taken at each release point: one near the stake, another about 50 feet north of the stake, a third 50 feet south, a fourth 50 feet east, and the last 50 feet west. Insects on 10 plants were counted for each sample once a week. The insects counted were greenbugs, greenbug mummies, corn leaf aphids, lady beetle larvae and adults, and other predators. No counts were made in the insecticide-treated fields since control of greenbugs was near 100%.

The counts of all insects were variable within plots and among replicates, as might be expected since there were different planting dates, different people making counts, and rapid counting so that all plots could be covered with the manpower available. Sometimes fields were too wet from irrigation for counts to be made on the scheduled date. Averages of part of the greenbug and mummy counts are given in table 6.

In the High Plains of Texas the first greenbugs appear on sorghum about the middle of May when plants are 6 to 8 inches tall. The origin of the initial infestation is difficult to determine. Sometimes greenbugs are on wheat and other small grains in the area and could transfer to sorghum. More likely, the initial infestation on sorghum comes from winged individuals transported northward by air currents. Johnsongrass along roadways often has infestations before there is colonization on sorghum, but in 1972, counts on this weed and early counts on sorghum failed to show any correlation. Regardless of the origin, there is only a gradual increase in greenbug numbers until the first part of July, when increases are rapid. This same pattern was prevalent in 1973 (table 6). By June 25–27 there were about 194 greenbugs per plant, but one week later there were about 1,010 per plant, an increase of over fivefold.

It is difficult to explain the differences in yield that were obtained on the basis of the number of

TABLE 5.—*Distribution of* L. testaceipes *adults 48 hours after release in sorghum fields, Lubbock, Tex., 1973*

Distance from release point (feet)	Adult parasitoids per plant							
	No. released per acre			Avg.	Direction from release			
	7,000	14,000	28,000		East	West	North	South
0	2.0	3.7	6.9	4.20	—	—	—	—
3	1.0	1.3	1.5	1.27	1.2	1.6	1.0	1.3
6	.6	.5	1.5	.87	.9	1.1	.8	.7
9	.3	.7	.6	.53	.7	.6	.4	.4
12	.3	.6	1.0	.63	.7	.8	.5	.5
15	.2	.1	.7	.33	.4	.3	.4	.2
18	.1	.2	.1	.13	.1	.1	.2	.1
21	0	.2	.4	.20	.1	0	.1	.1
24	.2	.4	.1	.23	.1	.1	.1	0
27	0	.1	.1	.07	0	.1	0	0
30	.1	0	.2	.10	0	.1	0	0
Avg.	.46	.71	1.19	.78	.42	.48	.35	.33

TABLE 6.—*Counts of live greenbugs and* L. testaceipes *mummies on sorghum in 1973*

Parasitoids released per acre[1]	No. insects per plant on— June 25–27	July 2–3	July 9–10	July 16–20	July 24–27	July 30–Aug. 1	Avg.
	Live greenbugs						
None	292	1,059	2,037	2,724	2,342	336	1,465
7,000 *L. t.*	207	1,295	2,348	2,748	2,522	490	1,602
7,000 *L. t.* + *A. a.*	149	910	1,945	2,539	3,617	316	1,443
14,000 *L. t.*	120	1,112	1,363	1,739	3,911	365	1,342
28,000 *L. t.*	200	674	1,369	1,517	3,553	593	1,262
LSD[2]	148	557	930	855	1,719	217	—
	Mummies						
None	0	1.3	1.3	10	977	940	322
7,000 *L. t.*	.3	4.3	5.7	84	1,458	1,535	501
7,000 *L. t.* + *A. a.*	0	2.0	7.0	91	1,552	1,540	532
14,000 *L. t.*	1.0	12.3	11.7	252	2,215	1,738	663
28,000 *L. t.*	1.3	5.7	15.0	260	1,694	1,547	582
LSD[2]	1.1	11.8	7.4	230	1,694	1,402	—

[1]*L. t.*=*Lysiphlebus testaceipes*, *A. a.*=*Aphelinus asychis*.
[2]LSD=Least significant difference at 0.05 level.

greenbugs or mummies among the parasitoid release plots and the control. Perhaps the releases of 14,000 and 28,000 *L. testaceipes* per acre kept the greenbugs from surpassing the number necessary to cause measurable damage to the sorghum for a longer period of time. For example, when no parasitoids were released the greenbugs increased to over 2,000 per plant as early as July 9 or 10. When 14,000 and 28,000 *L. testaceipes* per acre were released, this level of greenbugs was not reached until July 24–27, about 2 weeks later than the control and only about 1 week before the unexplained decline of the pest. Thus, there may be a time factor involved in greenbug damage to sorghum. Nevertheless, the number of greenbugs surpassed the economic threshold of about 1,500 greenbugs per plant in all the release treatments, and thus the plots could have been judged to need insecticide treatment.

An unexplained collapse of greenbug populations during the first week of August has occurred at least in 1970 to 1973. The disappearance of greenbugs is very rapid and widespread and involves plants at different stages of growth in a fairly large production area. There does not seem to be any correlation with climatic changes. A rapid decline of greenbugs on plants nearing maturity could be expected, but younger plants had very few greenbugs after the first week in August. The only enemy of the greenbug that seems sufficiently abundant to influence the population collapse is *L. testaceipes*.

The number of mummies in the various treatments followed roughly a reverse pattern of the number of greenbugs. There were fewer mummies in the control and in the 7,000 *L. testaceipes* release plots than in the 14,000 and 28,000 per acre release plots until near the end of July. As the season progressed the mummy counts became more difficult to make because the mummies remained attached to the plants even after emergence of adults. No effort was made to differentiate old from viable mummies since such a distinction would have required the examination of each mummy—an impossible task considering the size of the test. The number of mummies was not influenced by the direction from the release point. Also, there were about as many mummies per sample near the release point as in samples 50 feet away. These findings are in agreement with the counts determining the distribution of adults from the release boxes.

There was about one mummy for every three greenbugs at the July 24 and July 30, 1973, counts. Adult parasitoids were numerous. This relatively high parasitoid level could account for the collapse of the greenbug infestation since mummy counts in all plots averaged 2,300 per plant by August 5. It is very important that this peak did not occur until after the greenbugs had surpassed the economic threshold.

No distinction was made between mummified greenbugs and corn leaf aphids; however, observations indicated that there were few of the latter. The corn leaf aphid mummy counts were subjective since this insect often locates deep in the whorl of a plant, and we did not unroll all whorl leaves. Also,

corn leaf aphid infestations are spotty in a field, with individual plants having many cast skins, but adjacent plants had few, if any. The corn leaf aphid counts did not differ significantly among the treatments. They peaked on June 25–27 at about 191 per plant and then declined slowly until plants reached the boot stage, when infestations averaged only three per plant. The corn leaf aphid probably had little influence as a host of *L. testaceipes* in the test except that the adults may have fed on honeydew. Laboratory tests did not indicate that adult feeding was necessary.

Samples of approximately 100 greenbug mummies were collected near the release points in the fields during three different periods (table 7). Confined samples were returned to the laboratory and observed. The percentage of *L. testaceipes* emergence appeared low at first, but increased as the season progressed. Some observations indicate that moisture and relative humidity affect emergence, but a 50% emergence in the laboratory is considered satisfactory. The sex ratio was about 1:1, indicating good mating in the field. Secondary parasitoids increased to a high of near 15% by the time of the peak in the *L. testaceipes* population (July 24–27), but the effects upon the primary parasitoid efficiency may not have been great since the greenbug population collapsed.

TABLE 7.—*Emergence, sex ratio, and secondary parasitoids of field-collected* L. testaceipes *mummies*

	Dates Collected		
	July 12–13	July 25–26	August 2–3
Total mummies observed	3,015	10,784	6,463
Emergence (%)	44.6	76.4	77.4
Females (%)	50.7	48.6	51.6
Secondary parasitoids (%)	4.2	2.7	14.7

Aphelinus asychis will be briefly discussed, although the rearing of this parasitoid was not adequate to produce the 21,000 adults needed for release in the test. Such a large-scale rearing of *A. asychis* had not been undertaken before, and problems were expected.

A. asychis has a developmental period and sex ratio that compares favorably with those of *L. testaceipes* in laboratory tests (*7*). It readily parasitizes the greenbug (*1*), can be stored, and oviposits in aphid species other than the greenbug, thus being afforded an alternate host in case there is a scarcity of one (*9*). Although the mummies are black and would be easy to distinguish in the field after release, the favorable aspects of *A. asychis* were not sufficient for it to establish itself in our tests beyond the second generation, even though about 7,000 were released. The reasons for this failure are speculative.

The mummies containing *A. asychis*, unlike those containing *L. testaceipes*, drop from the plant. Cultivation and irrigation could have destroyed the dislodged mummies before adults could emerge. Also, *A. asychis* adults may not have been able to compete with *L. testaceipes* since observations indicated that the latter species was more active in exploring plants. *A. asychis* may have encountered unfavorable weather conditions. Even if *A. asychis* had continued beyond two generations, we do not know whether or not it could have effectively suppressed greenbug populations. Furthermore, the relationship of secondary parasitoids to *A. asychis* is unknown. There was little reason to be optimistic about *A. asychis* as a release parasitoid of the greenbug.

The lady beetle counts were erratic. Adult counts could be expected to vary considerably among the samples since this life stage is transient and its presence in a field is influenced by available cover, the time of day, and the intensity of sunlight. However, the larval counts were also highly variable. Perhaps larvae were sometimes located down whorls or behind leaf sheaths and thus were missed during counts. The overall counts of adults averaged slightly above 0.3 per plant on June 18–20 when greenbugs were about 18 per plant. They increased to only 1.8 per plant when greenbugs had increased to 3,189 per plant by July 30 to August 1. Larvae averaged 5.1 per plant by July 30 to August 1. This means that each adult and larva would have had to eat over 450 greenbugs in a day to have caused the collapse of the greenbug infestation. No doubt lady beetles consumed many aphids during the growing season, but they may have destroyed as many parasitized greenbugs as they did healthy ones. There were no significant differences in the number of lady beetle adults or larvae among the treatments.

Lady beetles were the only predators sufficiently numerous to have had an impact on the greenbug infestation, although five others were noted during the sorghum-growing season. The accumulative effects of these are unknown. Green lacewing adults increased to 0.3 per plant but not until late in the season.

HARVEST RESULTS IN RELEASE TEST

Harvest data were collected in each of the 18 plots of the release test 2 or 3 days before combining. This meant that the moisture content of the grain was about 15% to 16%. Five samples spaced about 100 feet apart were taken in each plot. Each sample consisted of 13.1 linear feet in each of two rows (1/500 of an acre). Sorghum heads were cut from plants, counted, and weighed in the field. Threshed grain weights were based on 70% of the head weights. A final stand count was made for comparison to earlier counts taken about 3 weeks after planting. The heads per acre, heads per plant, and threshed grain per head were calculated. A summary of the results is given in table 8.

Harvest values varied considerably, and many replicates were needed. Nevertheless, significant differences were obtained. The releases of 14,000 and 28,000 *L. testaceipes* per acre and the insecticide treatment had significantly higher yields than the control without insecticide or release. The previous two treatments also gave more grain per head (larger heads) and resulted in significantly fewer heads compared to the control. These results could be expected. Greenbug damage often causes small plants to die, and subsequently there is a reduction in stand. Stand counts in our test were erratic, but there was no obvious large stand loss since plants were well established before large numbers of greenbugs became established. Damage to larger plants tends to kill the main shoot, but the plant recovers sufficiently to produce one or more tillers, which usually have relatively small heads. The data and greenbug counts indicate that plants in our tests were infested in a late growth stage. Tillers frequently are late maturing, and this could delay harvest and subject the crop for a longer period to adverse weather, diseases, and insects. Also, tillers tend to be weak and may lodge.

Thus, there may be indirect benefits as well as grain yield increase from controlling greenbugs on grain sorghum. Whether insecticides, released parasitoids, or other methods are used may be decided on a monetary basis.

EVALUATION OF THREE METHODS OF GREENBUG CONTROL

Table 9 gives a rough estimate of the costs of producing, storing, shipping, and releasing of 28,000 parasitoids per acre. The cost would be several times higher than the cost for insecticidal treatment. Aerial release would lower the cost by about $2 per acre, but there is little hope of reducing the cost of labor for surveying fields before and after release. Releasing a larger number of parasites would not increase cost proportionally to the additional number of parasites, but we have no assurance that a release above 28,000 would give better control.

TABLE 9.—*Costs of producing, storing, shipping, and releasing 28,000 parasitoids per acre, 1973*

Cost item	Amount
Labor at $2 per hour:	
Planting, infesting	$14
Harvesting, packing, shipping	5
Releasing, monitoring	15
Total	34
Facilities, utilities	9
Equipment	10
Travel (10 miles at 10¢ per mile)	1
Total	$54

TABLE 8.—*Effect of released parasitoids of the greenbug on grain sorghum yield and stand*

Greenbug control treatment[1]	Grain/acre (lb)	Stand reduction[2] (%)	Heads/acre	Heads/plant	Grains/head (oz)
None	5,300	19	89,900	1.73	0.95
7,000 *L. t.* per acre	6,340	12	78,400	1.25	1.29
7,000 *L. t.* per acre + *A. a.*	6,470	8	89,000	1.40	1.16
14,000 *L. t.* per acre	6,940	9	85,900	1.24	1.29
28,000 *L. t.* per acre	7,600	10	81,000	1.06	1.50
Insecticide	7,880	13	74,800	1.02	1.69
LSD[3]	1,630	7	26,700	.58	.52

[1]*L. t.* =*Lysiphlebus testaceipes*, *A. a.* =*Aphelinus asychis*.
[2]Reduction based on the differences of stand counts before greenbugs became abundant and those at harvest.
[3]LSD=Least significant difference at 0.05 level.

TABLE 10.—*Comparison of insecticidal control, parasite release, and plant resistance during 1973 test period*

Item	Insecticide	Parasitoid release	Plant resistance
Cost per acre to grower.	$4.25 maximum; could be reduced to $2.50 by using less insecticide (1973).	$54 in 1973; could be reduced but not to the cost of insecticide.	No cost.
Scouting needed.	Up to time of treatment; repeat each year.	Up to time of release; then until greenbug population collapses.	None.
Greenbug control.	Good	Fair but variable	Good.
Effect on other insects.	Kills all, including beneficial insects; can lead to other pest problems.	Slightly controls corn leaf aphids.	None.
Environmental pollution.	Yes	No	No.
Commercial impact.	Requires scouts, aerial spray operators, chemical manufacturers and salesmen.	Would probably require more people than insecticide use.	Requires only plant breeders and seed companies; extra sales effort.
Restrictions	Clearance by regulatory agencies.	Regulations governing interstate shipment of live insects.	None.
Possibility of control breakdown.	Probably greatest; chance of greenbug developing resistance.	Probably least	Slight, since sources of resistance are varied.
Effort needed to to sell program.	Already in use	Extensive extension effort.	Some delay in starting, but acceptance should be rapid.
Compatibility with other control methods.	Affects biological control adversely.	Compatible with plant resistance but not insecticides.	Compatible with all.

Insecticidal control, parasite release, and plant resistance during the 1973 test period are compared in table 10.

SUMMARY

The early release of parasitoids was studied as a possible technique for the control of greenbugs on sorghum. *Lysiphlebus testaceipes*, which prefers greenbugs as a host and has a good reproductive and searching potential, was chosen as the major release insect. This well-adapted, native braconid was reared in large numbers on greenbugs grown on a mixture of barley and sorghum in aluminum flats and plastic pots. Infestation rates, rearing temperatures, infestation periods, harvesting periods, and harvesting techniques were some of the rearing procedures that were established during this study. Also, the establishment of storage procedures for the parasitoids permitted stockpiling until release dates. Cold-packing the insects for shipment by air prevented premature emergence. Parasitoids were placed in the field as mummies and allowed to emerge from the boxes in which they had been shipped. The boxes were attached 6 inches above ground level to stakes about 200 feet apart within field, with one release per 0.4 acre.

The parasitoids were released at 7,000, 14,000 and 28,000 per acre. Upon emergence the spread of adults was fairly uniform, and sampling of parasitoids showed little difference in counts at the release site and 50 feet away. Additional data showed that the higher mummy counts were found in the higher level release plots of 14,000 and 28,000 per acre until late July. At this point a high ratio (1:3) of *L. testaceipes* to greenbug occurred. This high ratio plus numerous uncounted adults probably caused the collapse of the greenbug infestation. The collapse, however, did not occur until after the population had surpassed the economic threshold. Native lady beetle counts during the test were erratic and showed no significant differences among plots.

Harvest results showed that the two higher release levels (14,000 and 28,000 per acre) and the insecticide plots produced significantly higher yields (pounds of grain per acre) when compared to the untreated control. These treatments also had

larger heads (more grain per head). The 28,000 release produced 7,600 pounds of grain per acre, compared to 7,880 pounds per acre from insecticide-treated plots, where almost 100% control of greenbug occurred. The no-treatment control produced 5,300 pounds per acre.

A comparison of control of greenbugs by insecticides, parasitoid release, and plant resistance indicates that the latter may be the most desirable technique. Plant resistance is now in use, whereas parasitoid release requires more study and resistance to insecticides has developed in greenbugs.

LITERATURE CITED

(1) Cate, R. H., Archer, T. L., Eikenbary, R. D., Starks, K. J., and Morrison, R. D. 1973. Parasitization of the greenbug by *Aphelinus asychis* and the effect of feeding by the parasitoid on aphid mortality. Environ. Entomol. 2: 549–553.

(2) Harvey, T. L., and Hackerott, H. L. 1969. Plant resistance to a greenbug biotype injurious to sorghum. J. Econ. Entomol. 62: 1271–1274.

(3) Hight, S. C., Eikenbary, R. D., Miller, R. J., and Starks, K. J. 1972. The greenbug and *Lysiphlebus testaceipes*. Environ. Entomol. 1: 205–209.

(4) Juneja, P. S., Gholson, R. K., Burton, R. L., and Starks, K. J. 1972. The chemical basis for greenbug resistance in small grains. 1. Benzyl alcohol as a possible resistant factor. Ann. Entomol. Soc. Am. 65: 961–964.

(5) Lingappa, S. S., Starks, K. J., and Eikenbary, R. D. 1972. Insecticidal effect on *Lysiphlebus testaceipes*, a parasite of the greenbug, at three developmental stages. Environ. Entomol. 1: 520–521.

(6) Peters, D. C., Wood, Jr., E. A., and Starks, K. J. 1975. Insecticide resistance in selections of the greenbug. J. Econ. Entomol. 68: 339–340.

(7) Raney, H. G., Coles, L. W., Eikenbary, R. D., Morrison, R. D., and Starks, K. J. 1971. Host preference, longevity, developmental period and sex ratio of *Aphelinus asychis* with three sorghum-fed species of aphids held at controlled temperatures. Ann. Entomol. Soc. Am. 64: 169–176.

(8) Rogers, C. W., Eikenbary, R. D., and Starks, K. J. 1972. A review of greenbug outbreaks and climatological deviations in Oklahoma. Environ. Entomol. 1: 664–668.

(9) Rogers, C. E., Jackson, H. B., Eikenbary, R. D., and Starks, K. J. 1972. Host-parasitoid interaction of *Aphis helianthi* on sunflowers with introduced *Aphelinus asychis*, *Ephedras plagiator*, and *Praon gallicum*, and native *Aphelinus nigritus* and *Lysiphlebus testaceipes*. Ann. Entomol. Soc. Am. 65: 38–41.

(10) Starks, K. J., Wood, Jr., E. A., Burton, R. L., and Somsen, H. W. 1975. Behavior of convergent lady beetles in relation to greenbug control in grain sorghum. Observations and preliminary tests. U.S. Dep. Agric., Agric. Res. Serv. [Rep.] ARS–S–53, 10 pp.

(11) Teetes, G. L., Schaefer, C. A., Gipson, J. R., McIntyre, R. C., and Latham, E. E. 1975. Greenbug resistance to organophosphorous insecticides on the Texas High Plains. J. Econ. Entomol. 68: 214–216.

(12) Ward, C. R., Huddleston, E. W., Owens, J. C., Hills, T. M., Richardson, L. G., and Ashdown, D. 1972. Control of the Banks grass mite attacking grain sorghum and corn in West Texas. J. Econ. Entomol. 65: 523–529.

(13) Weibel, D. E., Starks, K. J., Wood, Jr., E. A., and Morrison, R. D. 1972. Sorghum cultivars and progenies rated for resistance to greenbugs. Crop Sci. 12: 334–336.

(14) Wood, E. A., Jr. 1958. A hymenopterous parasite new to Oklahoma. J. Econ. Entomol. 51: 533.

(15) ——— and Starks, K. J. 1972. Effect of temperature and host plant interaction on the biology of three biotypes of the greenbug. Environ. Entomol. 1: 230–234.

ACKNOWLEDGMENTS

Appreciation is expressed to Albert Scheele, Lockney, Tex., and Roy Stence, Lubbock, Tex., sorghum producers who allowed us to use their fields for research.

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE
in cooperation with
The Agricultural Experiment Stations of Oklahoma and Texas

*1976-G.P.O.-1750/S/671-583/37